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Survival of Enteric Viruses on Environmental Fomites

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The survival of human enteric viruses on several porous (paper and cotton cloth) and nonporous (aluminum, china, glazed tile, latex, and polystyrene) environmental surfaces has been evaluated. Viruses persisted for extended periods on several types of materials commonly found in institutions and domestic environments. The stability of the viruses was generally influenced by environmental factors such as relative humidity (RH), temperature, and the type of surface contaminated. Overall, hepatitis A virus (HAV) and human rotavirus (HRV) were more resistant to inactivation than enteric adenovirus (ADV) and poliovirus (PV). The resistance to the desiccation step appears to be of major significance in determining the survival of a virus dried on fomites. ADV and PV showed a pronounced decrease in titer at this stage, whereas HAV and HRV displayed little decay at the desiccation step. HAV and HRV persistence was not affected by the presence of fecal material. On nonporous surfaces, PV and ADV persisted better in the presence of feces. However, on porous fomites the presence of fecal material had a negative influence on the survival of PV and ADV. Except for HRV, greater virus survival was observed at 4° than at 20°C. PV and HAV survival was enhanced at high RH; the survival of the latter was enhanced at least for nonporous materials. When dried on porous materials, HRV also exhibited greater persistence at high RH. The survival of ADV was not affected by RH. The validity of using bacteriophages of *Bacteroides fragilis* as indicators of human viruses dried on fomites was evaluated. *B. fragilis* phages persisted consistently longer than PV and ADV and sometimes survived as long as HAV and HRV.

Outbreaks of hepatitis A and acute gastroenteritis are a matter of frequent concern to the general community and to institutions such as day care centers, hospitals, nurseries, schools, and military quarters (1, 9). These viruses are excreted in high numbers in the feces of infected individuals (11), where they are able to persist for extended periods (31).

The transmission of human enteric viruses through contaminated drinking water, seafood, and fomites is a public health concern (26). In many outbreaks caused by enteric viruses, vehicular transmission of the agents apparently occurs via fecally contaminated environmental surfaces (7, 30). Although the behavior of poliovirus, the prototypical enteric virus, on environmental surfaces has been studied (12), data on the persistence of other enteric viruses of major health significance, such as hepatitis A virus, rotavirus, or enteric adenovirus, when dried on environmental fomites are scarce (18, 21). We report the comparative survival of human rotavirus, hepatitis A virus, enteric adenovirus, and poliovirus on representative porous and nonporous materials under different environmental conditions. The validity of using bacteriophages of *Bacteroides fragilis* as indicators of human viruses under these circumstances was evaluated.

MATERIALS AND METHODS

Viruses and cell cultures. Poliovirus 1 (strain LSc 2ab) (PV) and human rotavirus Ito^r p13 (HRV) were propagated and assayed in BGM and MA-104 cells, respectively, as previously described (3, 4, 14). FRhK-4 cell cultures were used to propagate and assay the cytopathogenic HM-175 strain (courtesy of T. Cromeans, Centers for Disease Control and Prevention, Atlanta, Ga.) of hepatitis A virus (HAV) (8). Human enteric adenovirus type 40 (ADV) (courtesy of W. O. K. Grabow, University of Pretoria, Pretoria, South Africa) was cultivated and assayed in CaCo-2 cell monolayers (25). Bacteriophage B40-8 of *B. fragilis* was assayed as previously described (33). The preparations of viruses used in these studies were deliberately not purified so that preparations as natural as possible were presented, as recommended by other authors (21).

Experimental design for survival studies. Viruses suspended in phosphate-buffered saline (PBS) or in a 20% fecal suspension (FS) were applied to fomites. Feces obtained from a healthy man were previously mixed with PBS, autoclaved, vortexed, and clarified by centrifugation at 1,000 × *g*. The materials chosen for this study were usually found in domestic and health care facilities, with the sole exception of polystyrene.

Two different kinds of fomites were distinguished according to their porosity: porous materials used were paper and cotton cloth, and nonporous materials used were aluminum, china, glazed tile, latex, and polystyrene. Nonporous materials, except latex and polystyrene, were thoroughly washed with tap water and disinfected with 70% ethanol prior to their use. Polystyrene was pretreated with 0.1% Tween 80, rinsed in sterile distilled water, and air dried (31). Latex was employed without any pretreatment.

Three sets of environmental conditions were used: 4°C with high relative humidity (HRH), 20°C with HRH, and 20°C with moderate RH (MRH). The high levels of RH were achieved and maintained by placing ultrawet paper inside a closed chamber at 4 or 20°C. The moderate level of RH was obtained by placing a mixture of $CaCl_2$ and silica gel inside a strongly closed plastic chamber. The RH levels used were 90% ± 5% at 4°C, 85% ± 5% at 20°C, and 50% ± 5% at 20°C. The air temperature and RH levels were monitored four times a week.

Each virus suspension (20 μl) in PBS or FS was added to 24-well plates containing pieces of approximately 1 cm^2 of paper, cotton cloth, aluminum, and latex and allowed to dry (3 to 5 h in a flow cabinet, at room temperature and at a flow

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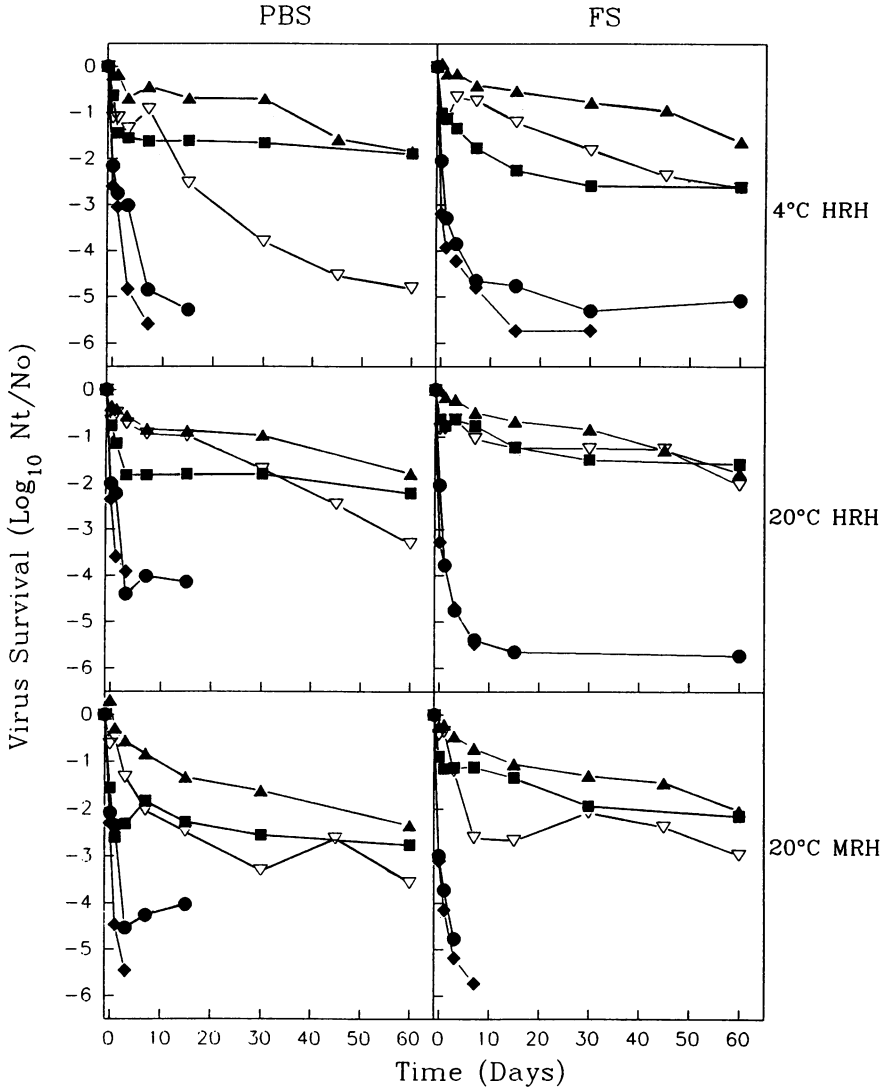

FIG. 1. Survival of human enteric viruses, expressed as $\log_{10} N_t/N_0$, dried on aluminum under different environmental conditions. ▽, B40-8; ▲, HAV; ■, HRV; ◆, PV; ●, ADV.

pressure of 20 mm). Because of their larger areas, china and glazed tile were inoculated with 50 μl and polystyrene wells were inoculated with 100 μl of each viral suspension. To evaluate the reduction in virus titer caused by the desiccation process, 20, 50, or 100 μl of each initial viral suspension (depending on the type of fomite) was diluted with 980, 950, or 900 μl, respectively, of 3% beef extract in saline; these solutions were used as controls. The plates were then covered, wrapped in aluminum foil, and placed at the desired temperature and RH. At designated times, two pieces of each surface inoculated with the different virus suspensions under the different ambient conditions were sampled. Viruses adsorbed to the fomites were eluted with 980 μl (paper, aluminum, cotton cloth, and latex), 950 μl (china and glazed tile), or 900 μl (polystyrene) of a solution of 3% beef extract in saline at pH 7.5, and after a 10-min contact time, each solution was vigorously pipetted 20 times to recover the sample (time zero). The eluates were stored at −75°C until assayed, except for an aliquot for phage assay that was kept at 4°C. Survival experiments were conducted over 60 days.

The survival of the viruses on fomites was determined by calculating the $\log_{10}$ of N_t/N_0, where N_0 is the initial virus titer and N_t is the titer at designated time intervals. Viral enumerations were performed by calculating the most probable number of cytopathogenic units per milliliter for infected cell monolayers grown in 96-well microtiter plates (25). Eight wells were infected for each dilution, and 10 μl of inoculum was added to each well. All samples from a given experiment were assayed at the same time and titrated at least in duplicate. Data were processed with a most-probable-number computer program (17). The analysis of variance test (32) was performed with log-transformed data to determine significant differences generated by the type of fomite, environmental conditions, and virus strain.

RESULTS

Virus survival on fomites. Human enteric viruses were able to persist for extended periods on fomites. Different patterns of virus behavior related to the type of environmental surface under study were obtained when viruses were dried on (i)

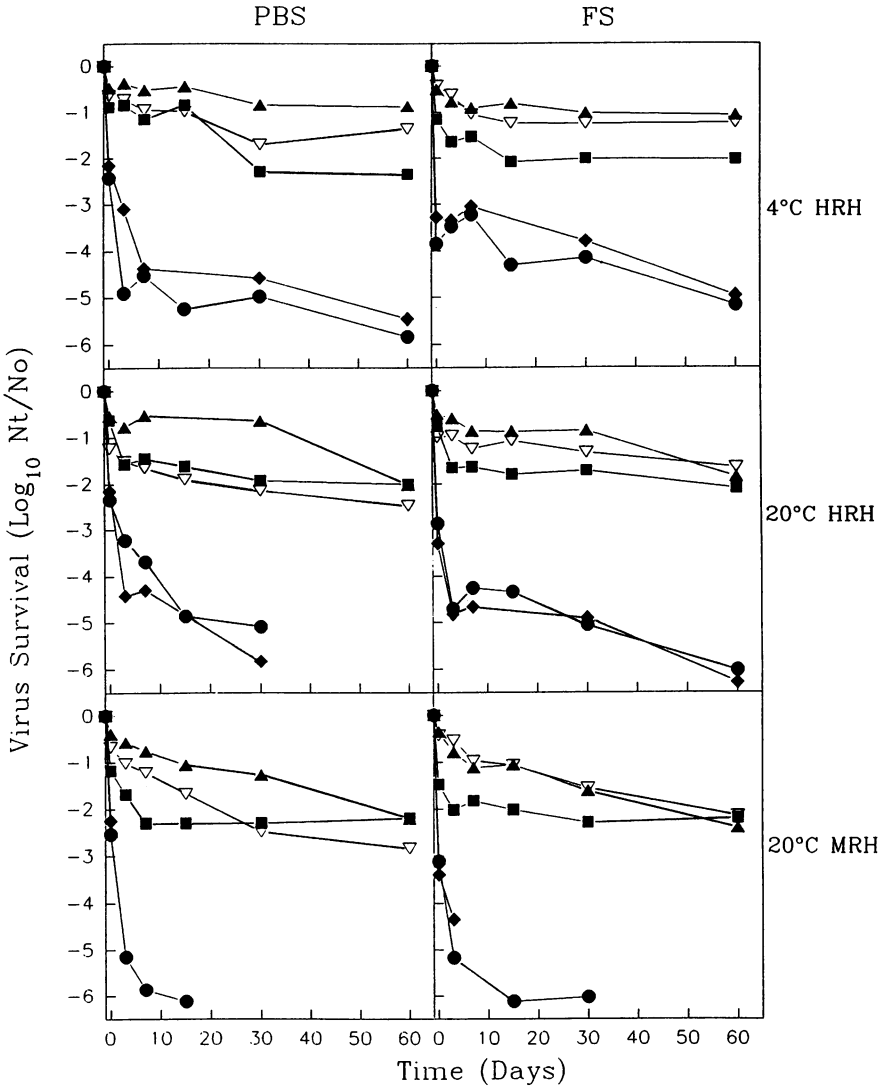

FIG. 2. Survival of human enteric viruses, expressed as $\log_{10} N_t/N_0$, dried on china under different environmental conditions. ▽, B40-8; ▲, HAV; ■, HRV; ◆, PV; ●, ADV.

aluminum, polystyrene, china, or glazed tile, (ii) paper or cotton cloth, and (iii) latex. For the sake of clarity, only results obtained with aluminum (Fig. 1), china (Fig. 2), latex (Fig. 3), and paper (Fig. 4) are shown.

As a general rule, HAV and HRV persisted longer than ADV and PV when dried on environmental fomites. On aluminum, HAV survived significantly ($P < 0.05$) longer than the other enteric viruses (Fig. 1). HRV was significantly ($P < 0.05$) more resistant than ADV and PV. PV was particularly susceptible to inactivation on aluminum under all assayed conditions.

Similar results were obtained when enteric viruses were dried on china (Fig. 2). However, ADV and PV showed greater survival capacities than on aluminum, but these values nevertheless were significantly ($P < 0.05$) lower than those for HAV and HRV. Similar data were obtained with glazed tile and polystyrene (data not shown).

On latex, HAV and HRV behaved similarly to each other, without significant ($P < 0.05$) differences between their survival capacities, and both viruses were much more ($P < 0.05$) resistant than PV or ADV (Fig. 3).

HRV showed greater ($P < 0.05$) persistence than HAV on paper (Fig. 4), except when it was suspended in fecal material at 4°C. PV and ADV did not significantly ($P < 0.05$) differ from one another in their inactivation rates, although these two viruses were less ($P < 0.05$) resistant than HRV and HAV. The patterns of virus behavior observed when viruses were dried on cotton cloth and paper were very much alike.

Effect of desiccation process on virus persistence. The desiccation step produced by itself substantial and differential levels of inactivation of human enteric viruses dried on fomites in the absence (Table 1) or the presence (Table 2) of fecal material. PV and ADV were the most susceptible strains, showing reductions in titer ($\log_{10} N_t/N_0$) ranging from −1.5 to −4.3, while the titer reductions of HAV and HRV ranged from −0.1 to −1.6. HAV was the strain most ($P < 0.05$) resistant to desiccation on nonporous surfaces, followed by HRV, which outlasted PV and ADV ($P < 0.05$). On latex, HAV and HRV

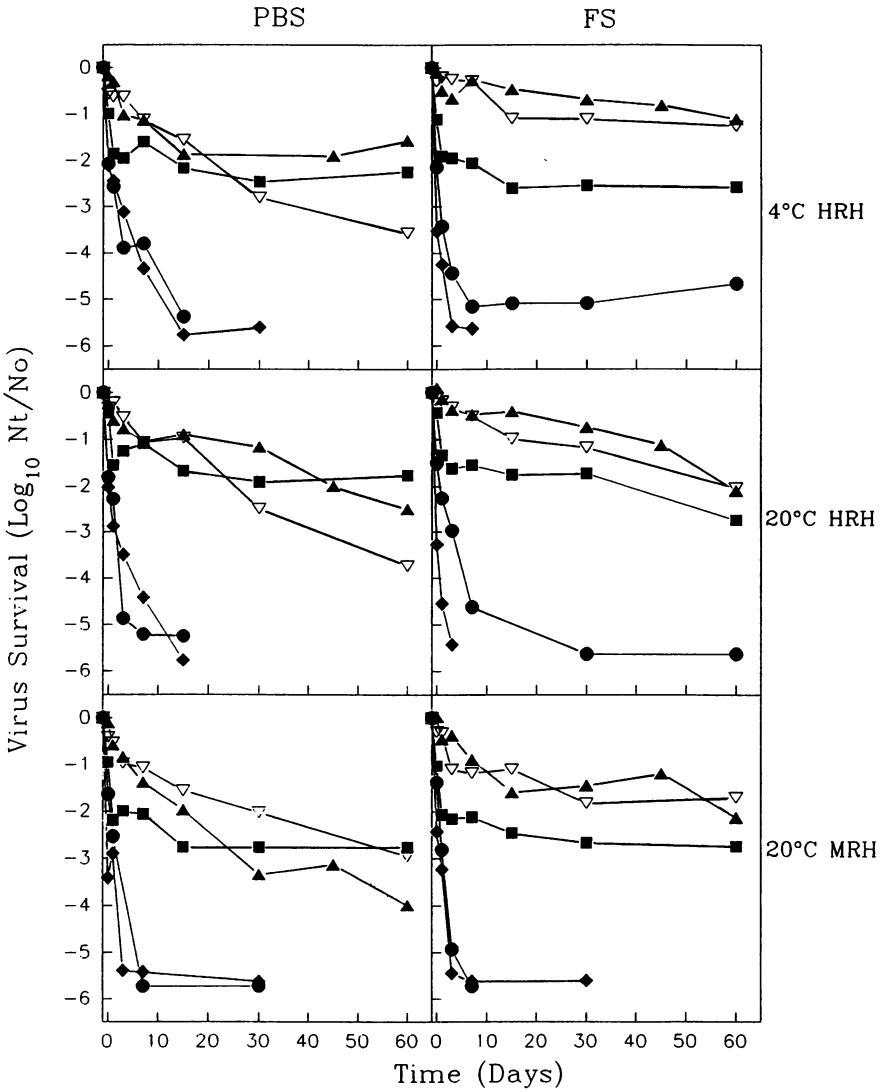

FIG. 3. Survival of human enteric viruses, expressed as $\log_{10} N_t/N_o$, dried on latex under different environmental conditions. ▽, B40-8; ▲, HAV; ■, HRV; ◆, PV; ●, ADV.

were significantly ($P < 0.05$) more stable after desiccation than ADV and PV. The latter strain was more ($P < 0.05$) susceptible to desiccation than ADV. On porous materials, HRV was the most ($P < 0.05$) persistent strain, followed by HAV, PV, and ADV, the latter of these being the most ($P < 0.05$) unstable virus.

Effect of fecal material on virus persistence. The effect of fecal material on the survival of viruses was paradoxical. HAV and HRV persistence was not affected by the presence of fecal material, although on latex (Fig. 3), HAV tended to survive longer when applied in a 20% FS. On the other hand, fecal matter affected the survival of ADV and PV differently depending on the nature of the fomite. On nonporous surfaces (aluminum, china, glazed tile, and latex) PV and ADV persisted longer in the presence of feces (Fig. 1 to 3), although without significant differences. However, on porous fomites (paper and cotton cloth) the presence of fecal material had a negative influence on virus survival, inducing a faster ($P < 0.05$) decay of PV and ADV (Fig. 4).

Effect of environmental conditions on virus persistence. With the exception of HRV, all enteric virus strains tested for their survival on fomites persisted longer at 4 than at 20°C (Fig. 1 to 4). The effect of low temperature on HAV and ADV survival was significant ($P < 0.05$) only after 1 month on porous materials and after 2 months on nonporous materials. In the case of PV, a greater ($P < 0.05$) survival capacity at 4°C was generally observed after shorter periods (0 to 15 days).

RH was also a factor influencing virus survival. HAV survival after 1 month was enhanced ($P < 0.05$) at HRH on nonporous materials, with the sole exception of china. HRV exhibited greater ($P < 0.05$) persistence at HRH when dried on porous materials after 1 month of desiccation. The survival of ADV was not affected by the RH level, and PV showed enhanced ($P < 0.05$) survival at HRH on nonporous materials, with the exception of latex, after 5 days.

Survival of bacteriophage B40-8 on fomites. When dried on nonporous fomites, bacteriophage B40-8 of *B. fragilis* survived longer ($P < 0.05$) than PV and ADV and similarly to HAV and HRV (Fig. 1 to 3). On porous materials, phage B40-8 survived for a shorter time ($P < 0.05$) than HAV and HRV. On these

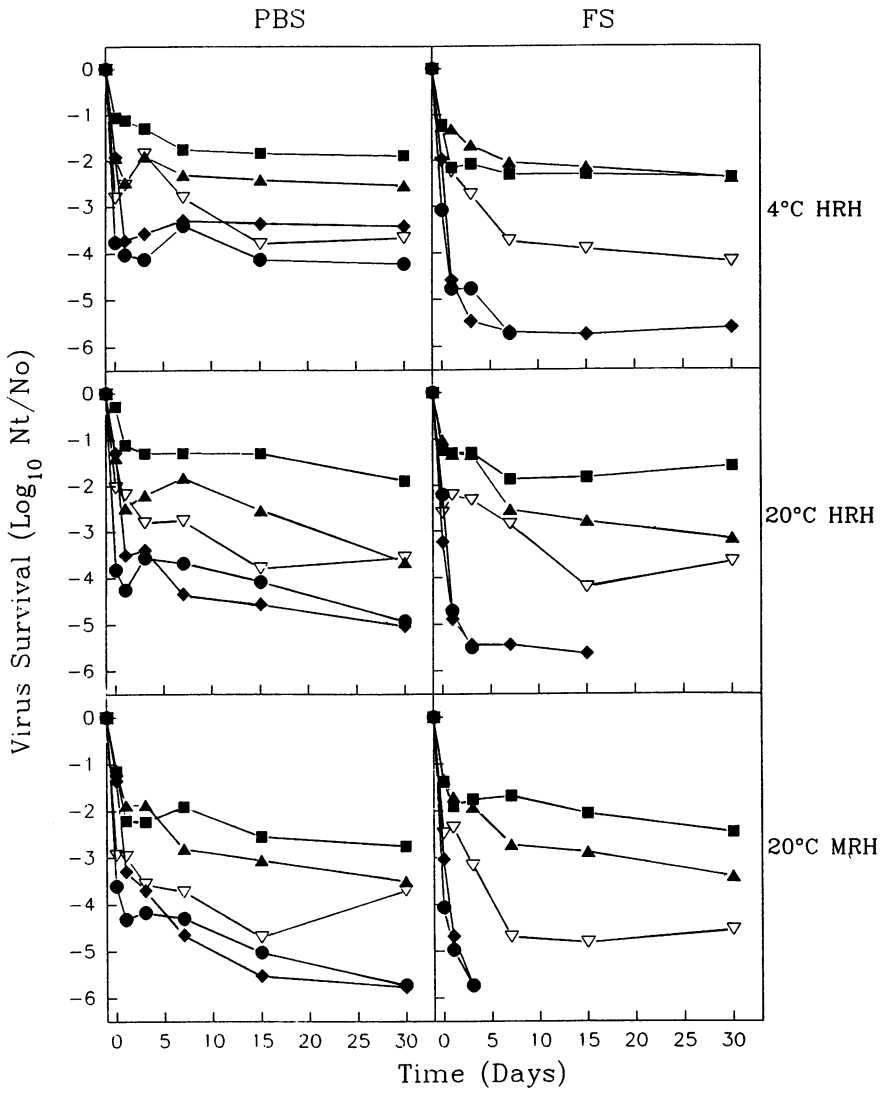

FIG. 4. Survival of human enteric viruses, expressed as $\log_{10} N_t/N_0$, dried on paper under different environmental conditions. ▽, B40-8; ▲, HAV; ■, HRV; ◆, PV; ●, ADV.

materials and after extended periods, B40-8 persisted longer ($P < 0.05$) than ADV and, under certain conditions, than PV (Fig. 4).

The level of inactivation of phage B40-8 by desiccation was dependent on the kinds of fomite and suspension medium. When applied to nonporous materials and suspended in PBS, B40-8 was inactivated more readily ($P < 0.05$) than HAV, as readily as HRV, and less ($P < 0.05$) readily than PV and ADV (Table 1). When B40-8 in PBS was dried on porous materials, only ADV was more ($P < 0.05$) susceptible to desiccation

TABLE 1. Reduction of infectivity of enteric viruses suspended in PBS after desiccation on fomites[a]

Fomite	Mean reduction of infectivity (SD)[b] of:				
	HAV	HRV	PV	ADV	B40-8
Polystyrene	−0.4 (0.1)	−1.2 (0.8)	−4.1 (0.4)	−3.1 (0.4)	−1.0 (0.7)
Aluminum	−0.1 (0.3)	−1.0 (0.5)	−2.4 (0.3)	−2.1 (0.4)	−0.7 (0.3)
China	−0.5 (0.2)	−0.9 (0.5)	−2.1 (0.1)	−2.4 (0.3)	−0.8 (0.3)
Glazed tile	−0.6 (0.2)	−1.2 (0.9)	−3.1 (0.3)	−2.3 (0.4)	−1.0 (0.3)
Latex	−0.2 (0.1)	−0.8 (0.3)	−2.4 (0.5)	−1.9 (0.4)	−0.5 (0.2)
Paper	−1.5 (0.8)	−0.8 (0.6)	−1.5 (0.4)	−3.7 (0.7)	−2.5 (1.1)
Cotton cloth	−1.6 (0.3)	−0.6 (0.3)	−2.7 (0.6)	−3.3 (0.5)	−2.9 (0.3)

[a] Virus suspensions were allowed to dry for 3 to 5 h in a flow cabinet.
[b] Reduction of infectivity is expressed as $\log_{10} N_t/N_0$.

TABLE 2. Reduction of infectivity of enteric viruses suspended in 20% fecal material after desiccation on fomites[a]

Fomite	Mean reduction of infectivity (SD) of[b]:				
	HAV	HRV	PV	ADV	B40-8
Polystyrene	−0.1 (0.2)	−0.9 (0.3)	−3.7 (0.5)	−4.3 (0.2)	−0.6 (0.5)
Aluminum	−0.1 (0.3)	−0.8 (0.4)	−3.2 (0.3)	−2.4 (0.6)	−0.8 (0.3)
China	−0.5 (0.3)	−1.1 (0.5)	−3.3 (0.3)	−3.4 (0.6)	−0.6 (0.3)
Glazed tile	−0.4 (0.1)	−0.3 (0.5)	−2.2 (0.6)	−3.5 (0.6)	−0.8 (0.1)
Latex	−0.1 (0.2)	−0.9 (0.3)	−3.1 (0.5)	−1.7 (0.5)	−0.3 (0.2)
Paper	−1.2 (0.4)	−1.3 (0.3)	−2.8 (0.8)	−3.1 (0.9)	−2.4 (0.4)
Cotton cloth	−0.8 (0.6)	−1.0 (0.5)	−3.5 (0.3)	−3.2 (0.7)	−2.2 (0.2)

[a] Virus suspensions were allowed to dry for 3 to 5 h in a flow cabinet.
[b] Reduction of infectivity is expressed as $\log_{10} N_t/N_0$.

(Table 1). When FS was used as the suspension medium, phage B40-8 was always more ($P < 0.05$) affected by desiccation than HAV, whereas it was inactivated less ($P < 0.05$) readily than ADV and PV on any type of fomite (Table 2). With respect to HRV, the behavior of B40-8 was variable depending on the kind of environmental surface (Table 2), being more ($P < 0.05$) susceptible to desiccation on nonporous materials.

The effect of fecal material on the persistence of B40-8 was evident only with nonporous surfaces, for which a strong ($P < 0.05$) protective effect by feces was observed (Fig. 1 to 4), except with aluminum at 20°C and MRH and china at 4°C and HRH, for which no significant differences were observed.

Temperature and RH do not seem to play significant roles in the survival of bacteriophage B40-8 when it is dried on environmental fomites (Fig. 1 to 4).

DISCUSSION

The results of this study clearly demonstrate that human enteric viruses are able to survive for prolonged periods on several types of materials commonly found in institutions and domestic environments. As a general conclusion, it can be stated that when dried on environmental fomites, HAV and HRV are more resistant to inactivation than ADV and PV. HAV has been reported to have an inherently more stable molecular structure than other picornaviruses, such as PV (36). With regard to HAV, inactivation rates obtained with porous materials were higher than those obtained with nonporous materials. For the other enteric virus strains tested, no significant differences based on the kind of fomite were detected, although PV and ADV persistence on porous surfaces was somewhat higher than on nonporous materials. Comparatively, PV, ADV, and the B40-8 phage persisted for a shorter time on aluminum than on any other nonporous material. It has been reported (35) that aluminum damages the proteins of poliovirus virions adsorbed onto the metal surface. Another conclusion drawn from our data is that polystyrene may be used as a model fomite in studies on the behavior of enteric viruses on nonporous environmental surfaces.

The resistance to the desiccation step appears to be of major significance in determining the ability of a virus strain to survive when dried on fomites. A pronounced decrease in titer at this stage, as has been observed for ADV and PV, dramatically reduces the chances of a subsequent virus persistence. On the contrary, HAV and HRV, which have been involved in outbreaks probably due to transmission via fecally contaminated environmental surfaces (20, 30), show little decay at the desiccation step.

In temperate climates, infections due to enteroviruses generally reach a peak in summer and early fall (24), whereas rotavirus infections occur mainly during the cooler months (22), although seasonal and nonseasonal distributions of rotavirus in sewage have been described (5, 16). On the other hand, cases of hepatitis A do not show a clear seasonal pattern (19). The few studies on the seasonality of enteric adenovirus infections report peaks of diarrhea in midsummer (37). All these data suggest that temperature and RH may play meaningful roles in the seasonal distribution of outbreaks of disease caused by certain human enteric viruses (10). The experimental conditions selected in our studies may be considered representative of the natural environmental conditions encountered in temperate and tropical climates. The stability of the viruses was generally influenced by environmental factors such as RH, temperature, and the type of surface contaminated. Laboratory studies have shown that, essentially, viruses persist better in the environment at HRH and low temperatures (6, 29, 31). Except for HRV, enhanced virus survival at a low temperature was generally observed in our experiments. HAV was reported to survive longer when RH was low or moderate (21). However, in our studies, conducted over much longer periods, HAV survival was enhanced at HRH, at least on nonporous materials. Previous data on the effect of RH on rotavirus survival are contradictory: Moe and Shirley (23) showed that a field strain of HRV could survive longer when RH was kept either low or high than when RH was medium, whereas Sattar and coworkers (30) reported that HRV survived for a shorter time at HRH. We observed that when dried on porous materials, HRV exhibited greater persistence at HRH. The reasons for the differential stabilities of enteric viruses at different RH levels and the disparities between different studies remain to be elucidated.

Since the fecal-oral route is the common means of enteric virus transmission, it is interesting to evaluate the effect of fecal material on the persistence of viruses on fecally contaminated fomites. Data obtained in this and other studies (18, 31) on the protective effect of feces on viruses are contradictory: fecal matter affected the survival of enteric viruses in opposite ways, depending on the type of fomite and the virus strain.

The behavior of PV in these experiments poses a serious concern, since this virus has been extensively used as a model strain. From data generated in this and other studies (2, 21, 31), it can be concluded that PV does not provide an adequate indication of the behavior of human enteric viruses, such as HAV or HRV, in the environment under natural or disinfection conditions. As an alternative, bacteriophages have been proposed as indicators of viruses because their handling is simple and inexpensive and does not require specialized personnel or sophisticated facilities (15). In the present study, the survival of bacteriophages of *B. fragilis* (34) consistently exceeded that of PV and ADV and sometimes equalled that of HAV and HRV. *B. fragilis* bacteriophages are among the most

promising candidates to be used as substitutes for human viruses on fomites, particularly on nonporous surfaces.

Evidence gathered from some institutional outbreaks of enteric diseases suggests that surfaces may act as vehicles for the spread of infection (13, 27, 28). Keswick et al. (18) have suggested that the prevalence of asymptomatic infections in day care facilities may make them a reservoir of infection for previously uninfected children and their family contacts. Some enteric viruses have been shown in this study to survive on inert surfaces long enough to represent a source for secondary transmission of disease. However, it is generally very difficult to determine whether and to what extent fomites play a role in the spread of infectious agents. In any case, handwashing and proper sanitation and disinfection measures, especially in high-risk settings such as day care centers, hospital wards, and restaurants, should not be neglected.

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